Cryosurgical depigmentation of gingiva and its comparison with bur abrasion.

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CERTIFICATE

This is to certify that this dissertation titled **"Cryosurgical depigmentation of gingiva and its comparison with bur abrasion"** is a bonafide record of work done by Dr**. Vikas** under our guidance and to our satisfaction during his postgraduate study period of 2009-2012.

The Dissertation is submitted to The Tamil Nadu Dr. MGR Medical University in partial fulfillment for the award of the degree of Master of Dental Surgery – Periodontics, Branch II. It has not been submitted (partial or full) for the award of any other degree or diploma.

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DECLARATION

TITLE OF DISSERTATION	Cryosurgical depigmentation of gingiva and its comparison with bur abrasion
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LIST OF GRPAHS

A smile expresses a feeling of joy, success, sensuality, affection and reveals self-confidence and kindness. A smile is more than a method of communication and is a means of socialization and attraction. The harmony of the smile is determined not only by the shape, the position and the color of the teeth but also by the gingival tissues. Gingival health and appearance are essential components of an attractive smile[62].

Melanin is seen in the skin of people all around the world. However, the amount varies considerably among people of different races. The amount of pigment may also vary within same individual in relation to amount of exposure, the different body parts have had to actinic rays of sun[78].

The color of gingiva is determined by several factors, including races, vascularity, epithelial thickness, degree of keratinization, and pigments within the epithelium, melanin deposition is mainly located in the basal and suprabasal cell layers of epithelium[28].

Increasing demand of gingival esthetics has become a significant aspect of dentistry, so clinicians are often faced with achieving improved gingival esthetics. The color of gingiva that may cause esthetic concerns is an essential part of overall esthetics parallel to today's high cosmetic expectations[5].

Since there has been no single etiological factor suggested for hyperpigmentation of the gingiva, the condition poses a therapeutic challenge.

Many attempts have been made in the past to answer this cosmetic demand and eliminate these dark patches of pigmentation on the facial aspects of gingiva. Procedures which include non-surgical and surgical techniques like split thickness epithelial excision, free gingival grafts, cryosurgery, bur abrasion, laser therapy etc.

There are various methods mentioned in literature regarding gingival depigmentation but, information regarding repigmentation following surgical removal of pigmented gingiva in humans is limited. In order to find a better approach for gingival depigmentation, a comparative evaluation of surgical bur abrasion and cryosurgery was undertaken in this study.

1) To evaluate the efficacy of bur abrasion and cryosurgery for gingival melanin depigmentation.
2) To compare the rate of repigmentation, following bur abrasion and cryosurgery.

Oral Pigmentation

Colour of oral mucosa is the net result of number of factors, one of which is pigmentation. Two types of pigmentations occur: Endogenous, arising in tissues from normal physiologic processes, and Exogenous, caused by foreign material introduced into the body locally or systemically. The endogenous pigments most commonly contributing to colour of oral mucosa are melanin, hemoglobin and bile[54]. While melanin pigmentation is the most familiar, other pigments such as carotene, oxyhemoglobin and reduced hemoglobin may also contribute to the normal colour of integument and are also found in the masticatory mucosa[31].

Melanin

Melanin, a non hemoglobin derived brown pigment, is the most common of the endogenous pigments[58]. Melanin is produced by the specialized pigment cells called melanocytes, which are situated in the basal layer of the oral epithelium and the epidermis. These cells are derived embryologically from the neural crest ectoderm and enter the epithelium at about the eleventh week of development, there they divide and maintain themselves as a self-reproducing population. Melanocytes lack desmosomes and tonofilaments but possess long dendritic processes that extend between the keratinocytes, often passing through several layers of cells. Melanin pigment is synthesized within the melanocytes as small structures called melanosomes.

These are inoculated or injected into the cytoplasm of adjacent keratinocytes by the dendritic process of the melanocyte.

Melanin is synthesized from tyrosine and dihydroxyphenylalanine (DOPA) via dopaquinone by the oxidation of tyrosinase. When a section of heavily pigmented tissue is stained with hematoxylin and eosin and viewed under the light microscope, groups of melanosomes are identified and these groups are referred to as melanin granules.

Lightly and darkly pigmented individuals have the same number of melanocytes in any given region of skin or oral mucosa; colour differences results from relative activity of melanocytes in producing melanin and from the rate at which melanosomes are broken down in the keratinocytes. In individuals with very heavy melanin pigmentation, cells containing melanin may be seen in the connective tissue.

These cells are probably macrophages that have taken up melanosomes produced by melanocytes in the epithelium and are sometimes termed melanophages. The regions of oral mucosa where melanin pigmentation is seen most commonly are gingiva, buccal mucosa, hard palate and tongue. Despite considerable individual variation, a direct relationship tends to be seen between the degrees of pigmentation of skin and in oral mucosa. Light skinned persons rarely show any oral melanin pigmentation.

Melanocytes are involved in development of several pigmented lesions in oral mucosa. Oral melanotic macule appears clinically similar to a freckle and microscopically shows increased production of melanin pigment without proliferation of melanocytes. The condition is harmless. A nevus (mole) is a benign proliferation of melanocytes in oral cavity and it is not easy to distinguish clinically from melanoma. Melanoma is a malignant tumor of melanocytes. In oral cavity melanoma is rare but usually fatal. Treatment is surgical removal[54].

Epidemiology

Dummett CO[18] (1946) reported that gingival hyperpigmentation occurs as diffuse, deep purplish discoloration or as irregularly shaped brown and light patches. The distribution in blacks is as follows: hard palate - 61%, gingiva - 60%, mucous membrane - 22% and tongue - 15%.

Grosky M et al[34] (1984) reported the prevalence of physiologic gingival pigmentation in three ethnic groups of 2,465 Israeli Jews. The distribution of this gingival pigmentation was 57.9% of Eastern group, 43.9% of Sephardic group and 21.1% of Ashkenazi group. No indication of sex predilection was found. The most common site of pigmentation was the labial part of gingiva, and the attached gingiva was the most common pigmented anatomic division. The group of eastern division showed the highest prevalence of physiologic pigmentation in each of anatomic parts and division of gingiva.

Amir et al[3] (1991) stated all kinds of oral pigmentation appear in young children. In a study where 1300 children's were examined, oral racial pigmentation was identified in 13.5%. **Prinz**[64] (1932) on the other hand, claimed oral physiologic pigmentation did not appear in children and was clinically visible only after puberty.

Hedin CA and Axell T[36] (1991) interviewed 234 and 233 patients in Thailand and Malaysia respectively concerning tobacco chewing habits and examined for the presence of oral melanin pigmentation. It was found that tobacco smokers had more significant oral surface pigmented than non tobacco users.

It was concluded that tobacco smoking stimulates oral melanocytes to secrete more melanin.

Barrett AW and Scully C[8] (1994) reported that in Caucasians, the majority of melanocytes have striated granules that are incompletely melanized and vary in size from 0.1 µm to 0.3µm. Melanin is produced by these patients but it is insufficient to cause pigmentation, and less than 10% of Caucasians demonstrate gingival pigmentation. Greater amount of melanin granules are present in African-Americans and are more completely melanized. When pronounced clinical pigmentation is evident, the melanosomes form large complexes of 1 µm to 3 µm, and the size and degree of melanization of granules reflect the degree of gingival pigmentation and the degree of skin pigmentation.

Özbayrak S et al[58] (2000) in his study on intensity and distribution of racial pigmentation of the oral mucosa commented that oral mucosal pigmentation is variable, not only between races, but also between different individuals of the same race and within different areas of the same mouth. In darker skinned people oral pigmentation increases, but there is no difference in the number of melanocytes between fair-skinned and dark-skinned individuals. The variation is related to differences in the activity of melanocytes.

La Porta et al[47] (2005) reported a 45 years old, Caucasian female, presenting with pigmentation of gingiva, lips and nail bed. Past medical history revealed initiation of minocycline therapy 6 months earlier.

Histopathologic examination of biopsy specimen of gingiva showed increased evidence of melanin/ melanocytes in the epithelium and melanin/ melanophages in the connective tissue. Nine months after cessation of therapy, the patient exhibited marked reduction in pigmentation.

Hanioka T et al[35] (2005) in his study on relationship between melanin pigmentation in the gingiva of children and passive smoking performed on 59 nonsmoking children concluded that excessive pigmentation in the gingiva of children was associated with passive smoking.

Yousuf A[83] (2005) investigated the incidence of melanin pigmentation in 280 Bangladeshi individuals. He found the incidence of melanin pigmentation in smokers was 82% and in nonsmokers it was 58.3%. Melanin pigmentation occurred frequently in participants aged 20-49 years in smokers, while in non smokers it was observed in participants aged from 30-59 years. Gingiva and buccal mucosa were frequently involved sites in both the groups. The smokers group had tendency to show more intense melanin pigmentation than non smokers.

Rawal SY et al[65] **(**2007) reported about four black females (aged 19 to 56 years of West African origin (Mauritania and Senegal), representing three different ethnic groups (Fulani, Mandinka and Soninke) presented with various chief complaints. All exhibited diffuse pigmentation of the maxillary vestibular gingiva extending to the second premolar areas. The colour ranged from intense blue gray to light gray or grayish pink. One case was biopsied for histopathologic evaluation.

Questioning revealed that the women had one or more sessions of traditional gingival tattooing. In one case, the procedure was performed in a dental office. The color range appeared to depend on the time that elapsed since the last procedure. The biopsy exhibited dense fibrous connective tissue containing aggregates of foreign material consistent with a foreign body tattoo.

F Hajifattahiet al[24] (2010) conducted a study to evaluate the relationship between passive smoking and oral pigmentation in children. Participants were 400 healthy children, 10 to 11 years old who did not use any drugs. Pigmentation was seen in 150 children (75%) in the experimental group and 122 children (61%) in the control group (P<0.005). The relative risk of oral pigmentation for children who were exposed to passive smoking was 1.23. It was concluded that passive smoking can induce gingival pigmentation in children.

Clinical Characteristics

Becker SW[9] (1927) first isolated melanocytes in the oral epithelium; a few years later **Laidlaw** and **Chan**[45] **(**1932) isolated melanocytes from the samples of gingival tissues.

Patsakas A et al[59] (1981) reported the total number of melanophores in the attached gingiva was approximately 16 times greater than in the free gingiva.

Gorsky M et al[34] (1984) found the prevalence of gingival pigmentation was higher on the labial part of the gingiva than on the buccal and palatal/lingual parts of the arches.

Perlmutter S and Tal H[60] (1986) in his study of repigmentation reported that gingiva is the most frequently pigmented intraoral tissues. Microscopically, melanoblasts are normally present in the basal layers of the lamina propria. The most common location is the attached gingiva (27.5%) followed in decreasing order by the papillary gingiva, the marginal gingiva, and the alveolar mucosa.

Differential Diagnosis

Amalgam Tattoo

Buchner A and Hansen LS[13] (1980) reported the lesion which represents embedded amalgam particles and manifests itself as an isolated bluish or black macule in various areas of the mucosa. The color is usually described as black, blue, grey, or a combination of these. Almost half of these lesions were located on the gingiva and alveolar mucosa, the mandibular region being affected more than the maxillary region.

Argyria

Neville BW et al[56] (20002) reported that chronic exposure to silver compounds may occur as an occupational hazard or as a result of therapeutic use of silver compounds such as silver arsphenamine or silver nitrate. One of the earliest signs is appearance of slate-blue silver line along the gingival margins, arising due to deposition of silver and silver sulfide pigments. There is also diffuse bluish black discoloration of the oral mucosa.

Pigmented Nevi

Dyer PV and Eveson JW[22] (1993) reported pigmented nevi of oral cavity which are usually uncommon; he classified them as intramucosal, junctional, compound, or blue according to their histological features.

Scully C[68] (1999) reported the location of nevi, which are seen particularly on the vermillion border of the lips and the gingiva. They are usually grey, brown, or bluish macules and are typically asymptomatic.

Oral Melanotic Macules

Laskaris G[48] (1994) reported the oral melanotic macules, a relatively rare oral mucosal lesion, analogous to skin freckles, due to the focal increase of melanin production.

Mercado-Ortiz G[52] (1996) reported vermillion border of the lower lip, the most commonly involved site whereas, buccal mucosa, palate, and gingiva are less commonly affected.

Malignant Melanoma

Grinspan D et al[33] (1969) reported about Melanoma, a cancerous condition of the melanocyte. He suggested the presence of special corpuscles in this cell, known as melanosomes, which contain the necessary enzyme (tyrosinase) to transform amino acids into melanin.

Laskaris G[47] (1994) reported malignant melanoma of the oral mucosa affects both sexes equally usually after 40 years of age. The great majority of the lesions (about 70-80%) occur on the palate, maxillary gingiva and alveolar mucosa. Initially there usually is a solitary small asymptomatic brown or black macule.

Peutz-Jeghers Syndrome

Gorlin RJ et al[32] (1990) reported the clinical manifestations of Peutz-Jeghers syndrome, it manifests itself as freckle like macules about 1 to l0 mm in diameter on hands, perioral skin, and intraorally to include the gingiva, buccal, and labial mucosa.

Laskaris G[48] (1994) reported Peutz-Jeghers syndrome (intestinal polyposis) as a genetic disorder characterized by mucocutaneous pigmentation and hamartomas of the intestine.

Smoker's melanosis

Dummett CO[21] (1979) reported smoker's melanosis as a benign focal pigmentation of the oral mucosa.

Araki S et al[4] (1983) reported that it tends to increase significantly with tobacco consumption.

Laskaris G[48] (1994) reported that smoker's melanosis clinically, presents as multiple brown pigmented macules less than 1 cm in diameter, localized mainly at the labial surface of anterior attached gingiva and the interdental papillae of the mandible. Smoker's melanosis is more common in females usually after the third decade of life.

Antimalarial Drugs

Giansanti JS et al[30] (1971) described the appearance of antimalarial drug induced pigmentations as slate-grey in color, bearing some resemblance to pigmentation caused by silver arsplenamine.

Birek C and Main JH[11] (1988) reported various antimalarial drugs were capable of inducing intraoral melanin pigmentation. These drugs include: quinacrine, chloroquine, and hydroxychloroquine.

Minocycline

Cockings JM and Savage NW[17] (1998) reported minocycline responsible for soft tissue pigmentation. It is usually seen as brown melanin deposits on the hard palate, gingiva, mucous membranes, and the tongue.

Heavy Metals

Carranza FA and Saglie FR[15] (1990) reported that use of heavy metals absorbed systemically from therapeutic use or occupational environments may discolor the gingiva and other areas of the oral mucosa. Bismuth, arsenic, and mercury produce a black line in the gingiva which follows the contour of the margin. Lead results in a bluish red or deep blue linear pigmentation of the gingival margin (Burtonian line).

Exposure to silver causes a violet marginal line, often accompanied by a diffuse bluish-grey discoloration throughout the oral mucosa.

Addison's Disease

Chuong R and Goldberg MH[16] (1983) reported Addison's disease or primary adrenocortical hypofunction is due to adrenocortical damage and hypofunction. They found bronzing of the skin and increased pigmentation of the lips, gingiva, buccal mucosa, and tongue. They advocated oral pigmentation might be the first sign of the disease. A biopsy of the oral lesions showed acanthosis with silver-positive granules in the cells of the stratum germinativum. Melanin is seen in the basal layer.

Periodontal Diseases

Wrigt JM[81] (1984) reported that pigmentation is worsened by gingivitis, which increases vascular permeability and allows the heavy metals access to the soft tissues.

Bergamaschi O et al[10] (1993) reported about melanin re-pigmentation, which appears after surgical injury.

Hemachromatosis

Frantzis TG et al[26] (1972) reported hemachromatosis (bronze diabetes) as a chronic disease characterized by the deposition of excess iron (ferritin and hemosiderin) in the body tissues, resulting in fibrosis and functional insufficiency of the involved organs.

Laskaris G[48] (1994) reported hyperpigmentation both in skin and mucous membranes associated with hemachromatosis (oral and conjunctiva). The oral mucosa shows diffuse homogeneous pigmentation of gray-brown or deep brown in about 20% of the cases. The buccal mucosa and the attached gingiva are the most frequently involved sites.

HIV Infection

Langford A et al[46] (1989) reported patients infected with Human Immunodeficiency Virus (HIV), progessive hyperpigmentation of the skin, oral mucosa, fingernails, and toenails was reported being related to primary adrenocortical deficiency and to Zidovudine (azidothymidine) therapy.

Management of Gingival Pigmentation

Gingival depigmentation is a periodontal plastic surgical procedure whereby the gingival hyperpigmentation is removed or reduced by various techniques:

Chemical Agents

Hirschfeld I and Hirschfeld L[37] (1951) used a mixture of phenol (90%) and alcohol (95%) to burn out pigmented gingiva. The growth of new gingiva was prolonged, and repigmentation soon developed in three patients and the rest with the same results in short while. These substances caused tissue necrosis in addition to pain. The treatment was not acceptable to the clinician or the patient.

Gingivectomy Procedure

Dummett and Bolden[20] (1963) used gingivectomy to remove pigmented gingiva. Incisions were made so as to remove as much as clinically pigmented tissue as possible and surgical pack placed. They concluded that gingival resective procedures, if performed solely for cosmetic reasons, offer no permanent results. This procedure resulted with prolonged healing by secondary intention, excessive pain and discomfort caused by exposure of underlining bone.

Bergamaschi O et al[10] (1993) reported five white patients with comparable gingival pigmentation who underwent gingivectomy to remove bandlike melanin pigmentations for cosmetic reasons. Biopsy specimens were taken from gingivectomy sites and healing areas 2, 3, 6, 7, 15, 50, and 180 days and 1.5, 3, and 5 years after the procedure. Transmission electron microscopic study revealed melanocytes in the process of migration and undergoing mitosis 6 and 7 days postoperatively. Clinically, the intensity of the pigmentation varied among the patients. Two patients reached baseline coloration 1.5 years postsurgery, while three returned to baseline coloration by 3 years postsurgery.

De-epithelialization Techniques

Perlmutter S and Tal H[60] (1986) conducted a study where pigmented keratinized gingiva was removed in two Jewish Yemanite adult males. After surgery, the exposed lamina propria was covered by a periodontal pack for 7 to 10 days. The tissues were then observed periodically for signs of repigmentation. Healing was uneventful and the surgically treated areas in both patients remained depigmented over the first 2 years. After 32 months, some pigmentation was found in one of the patients, and with the exception of two limited sites, the area was completely repigmented after 7 years.

The surgically treated area in the second patient remained depigmented over an 8 year follow up period. They agreed with previous reports that the gingival repigmentation can occur spontaneously and suggested that further controlled studies to be undertaken to explore the biologic basis for repigmentation.

Farnoosh AA[25] (1990) used a high speed hand piece and surgical diamond bur to eliminate dark pigmentation in 20 patients. Slight repigmentation was observed in 2 cases after 20 months post surgical follow up. He concluded that since this technique is relatively simple, versatile and requires minimum time and effort. If repigmentation occurs, the procedure can be repeated in the same area without limitation or causing any permanent damage.

Bishop K[12] (1994) reported a case in which he performed gingival depigmentation using a large, round, diamond bur in a high speed handpiece with copious irrigation, with a continuous light brushing action. The de-epithelialized site healed well and no recurrence of pigmentation was observed for one year.

In a case report, **Jayaprasad K and Chava V**[40] **(**1999) described de-epithelialization technique by using a no 15 B.P. Blade and also used diamond bur with a high-speed hand piece. They concluded that the technique was very simple and did not require sophisticated and expensive armamentarium and that if repigmentation occurred later, the procedure could be repeated on the same area.

Almas K et al[2] **(**2002) used scalpel surgical technique for depigmentation of gingiva. His study showed that after surgical epithelial excision technique, healing was uneventful, patient's acceptance of the procedure was good and the results were excellent. There was no sign of repigmentation up to 6 months.

Nandakumar K and Roshna T[55] (2005) performed periodontal plastic surgery combining gingival depigmentation and esthetic crown lengthening in a single appointment using scalpel surgical technique. Crown lengthening by external bevel gingivectomy was completed initially. The gingivectomy was followed by the depigmentation procedure. Using a number 11 scalpel blade, the entire pigmented epithelium along with a thin layer of connective tissue (split thickness flap) was removed. This incision was carried out from the apical level of external bevel incision to the apical end of the attached gingiva (mucogingival junction) up to where the pigmentation extended. The area healed well after two weeks. A one-year follow up period did not demonstrate any tendency towards repigmentation of the gingiva.

Lawande S and Rao P[49] (2005) performed gingival depigmentation using a combination of scalpel and diamond burs. Desired results were achieved and maintained till 12 months, with no signs of repigmentation.

Prasad D et al[62] (2005) performed gingival depigmentation in three patients, in which for first patient gingival depigmentation using bur abrasion was performed for left quadrant and electrocautery was used for the right side and patients were followed for three months. For the remaining two cases epithelial excision was planned by scalpel technique. By the end of three months, side treated by bur abrasion showed slight recurrences of pigments than the other two methods.

Singh S et al[74] (2005) performed gingival depigmentation in one patient using 15 no. B.P. blade, partial thickness flap was raised in hyperpigmented areas. Patient was re-examined after 2 months, clinical evaluation revealed pleasing colour of gingiva, which was satisfactory from both clinician and patient's perspective.

Mokeem SA[53] (2006) performed gingival depigmentation in three patients using high speed handpiece and diamond bur (diameter of 2mm or 2.5mm). After 18 months, none of the cases showed any recurrences of the pigmentation.

Humagain M et al[39] (2009) performed gingival depigmentation in a 21 year old male patient. Scalpel technique was used for lower arch and bur abrasion was used for upper arch.

A partial thickness flap was elevated in lower anterior region and high speed handpiece and long fissure bur was used for upper arch. After one week healing was uneventful without nay post surgical complications. Gingiva appeared pink, healthy and firm giving a normal appearance.

Prasad SSV et al [63](2010) used a B. P. blade handle with a No.15 blade and a high speed hand piece with diamond bur was used to remove the pigmented layer. This study showed excellent results with no repigmentation up to 6 months.

Kanakamedala AK et al[41] (2010) used blade no.15, with B.P. blade handle to scrape the epithelium carefully with underlying pigmented layer. Satisfactory results were achieved and maintained till 6 months.

Kaur et al[43] (2010) performed gingival depigmentation of young individuals of age range between 17-30 years using kirkland knife starting from the distal aspect of last tooth of right side to the distal aspect of last tooth on the left side. Patients were followed up to 9 months. Out of 20 patients they reported that 15 patients exhibited repigmentation in 9 months.

Gokhale ST et al[29] (2011) compared the scalpel surgical technique with electrosurgery for gingival depigmentation. Electrosurgery was used for left segment of upper anterior and scalpel surgery was used for right segment of upper anterior. After removing the entire pigmented epithelium with scalpel, abrasion with bur was done to get physiologic contour of gingiva. No repigmentation was observed till 6 months.

Kathariya R, Pradeep AR[42] (2011) reported case series in which they performed gingival depigmentation using scalpel technique, bur abrasion and electrosurgery. No repigmentation was reported with scalpel technique till 24 weeks, no repigmentation was reported with bur abrasion till 12 weeks. Patient acceptance was not good with electrosurgery and results were not promising as compared to bur abrasion and scalpel technique.

Cryosurgery

Tal H. et al[76] (1987) conducted a study to test the effectiveness of cryosurgical destruction of gingival epithelium in the removal of gingival melanin pigmentation. A gas expansion cryoprobe, cooled to - 81°C was applied to the gingiva for 10 seconds. The treated gingiva remained depigmented during the follow up period of 20 months. He concluded that cryosurgery may prove to be the treatment of choice when gingival depigmentation is indicated.

Yeh C-J et al[82] (1998) applied liquid nitrogen with a cotton swab for 20 to 30 seconds in twenty patients with dark gingiva and were followed up to 2 years. No repigmentation was observed during the follow up period. It was concluded that this was a simple, bloodless procedure for depigmentation of the gingiva requiring no local anesthesia or sophisticated equipment.

A Darbandiand N Amel Shahbaz[1] (2004) in this study ten patients who had oral mucosal physiologic pigmentation. Gingival depigmentation was performed by applying nitrogen oxide (with the temperature of - 89.5°C) using a suitable probe of equal size of lesions, they were frozen for 20-30 seconds. After four weeks, all pigmented parts were cured and no recurrent lesion was observed in any of the patients till 6 months. They concluded that oral cavity is an ideal environment for cryotherapy and it can be used as an effective method for treating oral pigmentation and some other oral lesions.

Arikan F and Gurkan A[5] (2007) performed depigmentation of 21 patients with gingival melanin pigmentation using 1,1,1,2 Tetraflouroethane (TFE) and he concluded that there was significant difference between preoperative and postoperative measurements of pigmented areas. He also concluded that the use of TFE for cryosurgical treatment of gingival melanin depigmentation is practical and inexpensive. Moreover, unlike other cryosurgery methods no special equipment is required, and it is safe to store in the dental clinic.

Shirazi AS et al[73] (2010) performed gingival depigmentation on 15 healthy patients in anterior segments of both mandible and maxilla using a liquid nitrogen-cooled cotton swab for 2 times within 2 weeks. Achieved results were maintained up to 12 months.

Free gingival autograft

Tamizi et al[78] (1996) treated severe physiologic gingival pigmentation with an unpigmented free gingival autograft in 10 patients. No evidence of repigmentation was found after 4.5 years. Of the 10, only one exhibited repigmentation (after 1 year). Authors suggested, the use of free gingival graft on denuded bone for the treatment of esthetic problem in patients suffering from severe gingival melanin pigmentation.

Novaes AB Jr et al[57] (2002) used acellular dermal matrix allograft for the elimination of gingival melanin pigmentation. He used acellular dermal matrix allograft on right side of anterior maxilla and oral epithelium was removed from the contralateral side using diamond bur as aesthetic treatment of bilateral gingival melanin pigmentation. Patient was evaluated for 24 months postoperatively, signs of repigmentation began to appear after 6 months on the left side.

Phillips GE and John V[61] (2005) treated a localized pigmented lesion involving the interdental papilla, free gingiva, attached gingiva, and alveolar mucosa between teeth #6 and #7. A subepithelial connective graft was harvested from the patient's palate and trimmed to fit precisely into the recipient site. Healing was uneventful and patient was seen at 3 weeks and 2 months post-surgery.

Campbell CM and Deas DE[14] (2009) treated a female patient for a large amalgam tattoo located in alveolar mucosa on the facial aspect of her maxillary central incisors. Teeth #8 and #9 were involved after a traumatic childhood incident.

A two-stage surgical approach was used to eliminate the lesion, beginning with a subepithelial connective tissue graft to increase tissue thickness subjacent to the amalgam tattoo. After 6 weeks of healing, the overlying pigmented tissue was removed using laser surgery to expose the underlying grafted connective tissue. After 2 months of healing following laser surgery, the amalgam pigmentation was completely removed, with good colour match and an increased width of keratinized tissue at the surgical site.

Laser Irradiation

Trelles et al[79] (1993) treated melanotic spots in the gingiva with monoline 514 nm green light (1.5W, 300 milliseconds, O.5mm spot size) produced by an argon laser. The results reported that the restoration of the mucosa was optimal with excellent esthetic results.

Sharon E et al[70] (2000) conducted a study to know the efficacy of carbon-dioxide (Co_2) laser vaporization in ablating gingiva, oral mucosal and cutaneous melanin in dogs. Three dogs with pigmentation of the oral mucosa, gingiva and skin were recruited for the study. The procedure was performed by using 3W continuous-wave carbon-dioxide laser. Clinical and histologic examination showed carbon-dioxide laser to be effective in eliminating pigmented areas. No recurrence of melanin was detected in either the oral mucosa, or gingiva during the follow up period of 11 weeks. In the skin a small amount of melanin repigmentation was noticeable. It was concluded that carbon-dioxide laser surgery proved an effective tool for obliterating superficial melanin discoloration.

Atsawasuwan et al[6] (2000) reported the use of Nd: YAG laser for gingival depigmentation in 4 cases. The Nd : YAG laser was set at 6 Watts, 60 mill joules per pulse, and 100 pulses per second. They found no recurrence of pigmentation during the follow up period of 11 to 13 months. The authors concluded that Nd: YAG laser had shown to be a good option for gingival depigmentation and caution must be exercised in delicate areas such as marginal gingiva while using Nd: YAG laser.

Tal H et al[77] (2003) in this study 10 patient who requested cosmetic therapy for melanin pigmented gums. Treatment was carried out using an erbium:YAG laser. The laser beam was set at 500 mJ/10 pulses/second. The "brush" technique was applied until the gingival surface appeared clinically free of pigmentation.

Patients were observed for 6 months. He concluded that depigmentation of gingival melanin pigmentation by erbium:YAG laser radiation in a defocused mode was a safe and effective procedure. The esthetic results were pleasing and healing was uneventful.

Esen E et al[23] (2004) performed gingival depigmentation for 10 patients using superpulsed CO_2 laser (10 watts, 0.8mm spot size, 20 Hz, 10 milliseconds). Two cases of partial repigmentation were reported during 24 month follow up. It was concluded that superpulsed mode of CO_2 laser can be safe and effective method for elimination of gingival melanin pigmentation.

G Berk et al[28] (2005) used Er,Cr:YSGG hydrokinetic system laser set at 20 Hz, 1.75 W to 1.5W, with 20% to 40% air and 12% to 5% water spray for removal of pigmented gingiva in 2 patients.

The pigmented areas were treated in noncontact mode, and both cases were completed during one appointment. Both cases were performed without any anesthesia, no intra-operative or postoperative pain or discomfort appeared. After 24 hrs, the lased gingiva was partly covered with a thin layer of fibrin, which exfoliated during the first week following treatment. The ablated wound healed almost completely in 1 week. The results pointed out that YSGG laser is a good and safe choice for removal of pigmented gingiva without local anesthesia. The postoperative period is comfortable for the patient and healing is fast and good. No repigmentation occurred in either patient till 6 months.

Azzeh MM[7] (2007) performed laser ablation using erbium-doped:yttrium, aluminum, and garnet (Er:YAG) laser in 6 patients (settings: 250 mJ, 15 Hz, with water and air and using defocused mode without using topical or local anesthesia. Each patient required 20-25 minutes for the completion of treatment and follow up period ranged between 6 and 18 months. No recurrence was found during this period.

Rosa DSA et al[67] (2007) performed gingival depigmentation using erbium-doped:yttrium, aluminum, and garnet (Er:YAG) laser in 5 patients at 64.0 mJ/pulse and 10 Hz under water spray in contact mode. After 3 month evaluation, no gingival deformity or recession was observed, slight recurrence was seen in 1 case.

Suthprasetporn S[75] (2007) Two patients with gingival melanin hyperpigmentation were treated by 2780 nm Er,Cr:YSGG laser device which was set at 1.0-1.75 watt, 7% water and 11% air for gingival ablation, and then 0.5 watt, 0% water and 11% air for biologic bandage.

The operation was done in non contact mode under topical anesthesia. Patients were observed for 12 months. One patient showed slight repigmentation in the 11 month post op observation.

Mani A et al[50] (2009) performed gingival depigmentation in 1 patient with the combination of scalpel, diamond bur and Diode laser (wavelength 800-980 nm) satisfactory results were obtained and maintained for 3 months.

Ko HJ et al[44] (2010) treated three patients with melanin hyperpigmentation in the anterior parts of the gingiva were chosen for this case study. In the maxilla, the gingival deepithelization was conducted with a high speed diamond bur, whereas, in the mandible with a Nd:YAG laser. At the 4th week after the procedure, there was no repigmentation.

Ascorbic Acid for Gingival Depigmentation

Shimada Y et al[72] (2009) reported the effects of ascorbic acid on melanin formation; test was performed on B16 mouse melanoma cells and three-dimensional human skin models. In addition, a clinical trial was performed to investigate the inhibitory effects of a gel containing ascorbic acid 2-glucoside (AS-G gel) on gingival melanin pigmentation. This study used a double-masked, split-mouth design on 73 subjects with symmetric gingival melanin pigmentation. AS-G gel was applied to one side of the gingiva for 12 weeks, whereas placebo gel was applied to the other side as a control. Ascorbic acid significantly inhibited tyrosinase activity and melanin formation in B16 mouse melanoma cells.

The inhibitory effects of ascorbic acid on melanin formation were also significant in three-dimensional human skin models Moreover, in the clinical trial, a significant relative change in pigmentation was seen after 4 weeks with the application of AS-G gel compared to placebo.

Radiosurgery

Sherman JA et al[71] **(**2009) performed gingival depigmentation using radiosurgery. Melanin pigmented gingiva was touched with No. 135 ball shaped electrode or tapping the area with 134 L shaped electrode, power setting of 11 in fully rectified cut mode for thick gingiva and power setting of 7 partially rectified coag mode was used for softer consistency and areas close to alveolar mucosa. Satisfactory results were obtained and maintained up to 4 weeks.

Cryotherapy

Cryosurgery is that branch of therapeutics which makes use of local freezing for the controlled destruction or removal of living tissues[80].

History of cryotherapy[27]

The Egyptians used cold to treat injuries and inflammation as early as 2500 BC. Dominique-Jean Larrey, Napoleon's legendary surgeon, used it to facilitate amputations. Between 1845 and 1851 Dr. James Arnott of Brighton, England, used local cold application in the treatment of numerous conditions, including headaches and neuralgia. He used salt solutions containing crushed ice at a temperature of -18° to -24°C to freeze breast, cervical, and skin cancers.

The first clinical application of liquid air (-190°C) was in 1889 by a New York City physician, Campbell White, who used either a swab, a spray, or a brass roller device used for the treatment of diverse skin conditions, including lupus erythematosus, herpes zoster, chancroid, warts, and epitheliomas.

Solidified carbon dioxide (-78.5°C) was introduced into clinical use by Dr. William Pusey of Chicago which, was mainly used for the freezing techniques in dermatology. Liquid oxygen (-182.9°C) came into clinical use in the 1920s, but was unpopular because it was combustible.

Following World War II, liquid nitrogen (-196°C) became commercially available. In 1950, this cryogen was introduced into clinical practice by Dr. Ray Allington, who described the technique of using cotton swabs dipped in liquid nitrogen for the treatment of a variety of nonneoplastic skin diseases.

The Cryogens[80]

Cryogen is a substance used for cryosurgery. Over the years, several cryogens have been used. They include the following:

Cryogens and their effective temperatures

Table-1

Cryogen	Effective Temperature
1) Salt – ice	-20°C
2) $C0_2$ slush	-20°C
3) Fluorocarbons (Freons)	- 30°C
4) Nitrous oxide	-75°C
5) $C0_2$ snow	-79°C
6) Liquid nitrogen	-20°C (swab) -196°C (spray)

Definition of Cryosurgical Terms[66]

Freeze time

It is the time elapsed from the start to the end of the freeze cycle. It is the duration of cooling. As less necrosis is required for benign lesion than for malignant one, a short freeze time suffices. For larger tumors, proportionately longer freeze time is required. The freeze time is longer for the cryoprobe technique than the open spray and shortest with the closed cone technique.

Lateral spread of freeze

The lateral spread of freeze refers to the freezing of the tissue beyond the estimated margins of the lesions. For malignancies, it should extend at least 3-5mm or more beyond the tumor. In case of verrucae, it should reach 2-3mm.

Freeze-thaw cycle

The actual freezing and subsequent thawing is referred to as freeze-thaw cycle. A single cycle generally suffices the successful eradication of benign and pre malignant conditions. A double freeze thaw cycle is recommended for malignant lesions to ensure greater lethality.

1) **Dipstick Method**

Cotton tipped applicator is dipped in liquid nitrogen and applied firmly to the lesion until a narrow halo of white ice forms around the bud. This technique is suitable for treating superficial benign lesions only.

2) **Spray Technique**

This is the most popular method. The spray method employed may be spot freeze, paint brush spray method, spiral spray method or rotator spray method. Liquid nitrogen is poured from the storage container into the spray unit slowly, using a funnel, until the unit is filled up to 2 inches from the brim. After the lid is screwed back, one should wait for 3-4 mins for pressure to build up. The appropriate screw on brass spray tip is selected. K-Y jelly is applied to the lesion. The spray tip is held 1cm away and steady spray of liquid nitrogen is directed at the centre of the marked lesion. The ice field gradually extends up to the edge of the circle. The freeze time commences once the solid ice has formed over the entire marked area. The lesion is allowed to thaw slowly. It should be allowed to thaw completely before refreezing if a second freeze is required.

3) **Cryoprobe Technique**

In this technique, liquid nitrogen is circulated so as to cool the tip of the cryoprobe to be applied to the lesion. Hence, freezing occurs by conduction. A probe suitable for the lesion is to be treated is selected and is precooled before application of the surface of the lesion. Its tip is applied firmly to the lesion and cooling is commenced.

The probe is allowed to thaw sufficiently before removing it from the treatment site. A repeat cycle, if required, should be commenced after allowing the lesion to thaw completely.

4) Cryoroller Technique

This is similar to dipstick method. The metallic cylindrical end of roller is dipped in liquid nitrogen held in polystyrene, thermocol or plastic cup and then rapidly rolled over the surface of the lesion.

5) Cone Spray Technique

This is used to concentrate the spray and limit its lateral spread. A cone of sufficient skin surface size is chosen to encompass the field to be frozen. There is rapid rate of fall in temperature and therefore this method is more destructive than open spray method.

The Mechanism of Injury[80] Various mechanisms are responsible for cellular and tissue injury. These include:

1. **Ice formation:** Extracellular ice formation damages the cell membranes, while intracellular ice is thought to damage the mitochondria and endoplasmic reticulum.

2. **Osmolarity changes:** Extracellular ice formation is associated with a decrease in extracellular water and a resulting increase in solute concentrations. This brings about cell membrane disruption.
3. **Thermal shock**
4. **Denaturation of lipoprotein complex**
5. **Vascular changes:** There is ischemic necrosis that starts around the vessels as a result of microthrombi within the capillaries and arterioles.
6. **Cryoimmunomodulation:** There is some evidence that low temperature can induce effective immune recognition of the remaining viral or tumor cells.

The above mentioned mechanism are further dependent on several factors such as freezing, rate of thawing (rewarming), temperature achieved, type of tissue and its vascularity. Rapid freezing and slow thawing is more destructive than slow freezing and rapid thawing.

Indications[69]

1. Vascular lesions
2. Benign tumors
3. Acne
4. Pigmented lesions
5. Viral infections
6. Inflammatory dermatoses
7. Infectious dermatoses
8. Pre-malignant ant malignant tumors

Contraindications

Absolute Contraindications

1. Lesion for which tissue pathology is required (Biopsy must be performed before cryosurgery is considered).
2. Lesion located in an area with compromised circulation.
3. Cold intolerance
4. Blood dyscrasias of unknown origin
5. Raynaud's disease
6. Cold urticaria
7. Cryoglobulinemia
8. Sclerosing basal cell carcinoma or recurrent basal cell or squamous cell carcinoma, particularly when located in a high-risk area (e.g., temple, nasolabial fold).

Relative Contraindications

1. Lesions located in pretibial areas, eyelid margins, nasolabial fold, alanasi, and hair bearing areas.
2. Keloidal tendency
3. Collagen vascular diseases
4. Dark skinned individuals due to high risk of developing cosmetically protracted hypopigmentation
5. Patients with sensory loss at lesional sites
6. Pyodermagangrenosum

Complications

Acute complications

1. Local pain
2. Edema
3. Cryoblister formation
4. Syncope
5. Headache

Subacute complications

1. Hemorrhagic necrosis
2. Wound infection due to the use of infected cryoprobes or redipping cotton swabs in to the cryogen.
3. Delayed wound healing
4. Temporary scar hypertrophy
5. Subcutaneous emphysema due to insufflations of the underlying tissue on spraying over broken skin.

Protracted complications

Common

1. Hypopigmentation, especially in dark skinned individuals, which can be minimized by freezing for <30 s.
2. Localized hypoaesthesia due to nerve damage, especially in areas where nerve lies superficially, such as sides of fingers, angle of jaws, post-auricular areas, sides of tongue and ulnar fossa of elbow.
3. Milia formation
4. Cicatricial alopecia, which can be minimized by freezing by <30 s.

Uncommon

1. Cartilage damage
2. Traumatic neuroma
3. Pyogenic granuloma
4. Fibroxanthoma

Repigmentation

Dummett CO[19] (1959) "Oral repigmentation refers to the clinical reappearance of melanin pigment following a period of clinical depigmentation of oral mucosa as the result of chemical, thermal, surgical, pharmacological or idiopathic factors".

Perlmutter S and Tal H[60] (1986) conducted a study on repigmentation of the gingiva following surgical injury. Two patients who had moderate to heavily pigmented gingiva were treated. Surgically treated areas in both patients remained depigmented over the first 2 years. After 32 months, some pigmentation was found in one of the patients and with the exception of two limited sites. They found different degree of repigmentation after 7 years. The other subject revealed no repigmentation over an 8 years follow up period. The authors concluded that these observations agreed with previous reports that described gingival pigmentation as spontaneous. It was proposed that repigmentation occurred due to the migration of active melanocytes from the adjacent pigmented tissues, which migrate to the treated areas, causing repigmentation.

Farnoosh AA[25] (1990) performed gingival depigmentation using high speed handpiece and diamond bur in 20 patients and reported repigmentation in 2 cases after 20 months.

Bergamaschi O[10] (1993) performed gingival depigmentation in 5 patients using gingivectomy, two patients reached baseline coloration 1.5 years postsurgery, while three returned to baseline coloration by 3 years postsurgery.

Novaes AB Jr et al[57] (2002) performed gingival depigmentation in one patient using acellular dermal matrix allograft on right side of anterior maxilla and oral epithelium was removed from the contralateral side using diamond bur. Repigmentation began to appear after 6 months on the left side, while no repigmentation was seen on the right side till 24 months.

Holtzclaw D et al[38] (2009) showed repigmentation of palatal mucosa from which free gingival grafts were harvested for augmentation of keratinized gingiva around implants. Palatal donor sites healed with spontaneous pigmentation. It was concluded that during healing, activation of hypoactive melanocytes secondary to hypermitotic activity of the melanocyte housing basal layer may explain the donor-site pigmentation pattern observed. An additional explanation for this pigmentation healing pattern may be endothelin-1 (ET-1)-induced hyperpigmentation. ET-1 is a 21-amino-acid peptide with a number of functions, among which is the constriction of blood vessels. Upon blood vessel damage, intense ET-1 expression from endothelial cells, in addition to the release of a cornucopia of other factors such as thromboxane, bradykinin, and serotonin, produces vessel constriction in an attempt to gain hemostasis. ET-1 is also produced by keratinocytes, and its level of expression in gingival epithelial cells is increased during inflammation.

After the excision of gingival tissue, healing of the wounded area occurs via initial hemostasis and subsequent formation of inflammatory granulation tissue over which epithelial cells migrate from surrounding wound margins.

The harvesting of free gingival grafts from palatal tissues results in a significant amount of microscopic vascular damage and inflammatory healing, which may upregulate ET-1. Such an upregulation may lead to hyperpigmentation, as ET-1 is a proven stimulator of melanocyte proliferation.

Kaur et al[43] (2010) reported repigmentation in 15 out of 20 patients over an observation period of 9 months after de-epithelialization using Kirkland's gingivectomy knife. Seven patients showed no repigmentation at all.

They reported repigmentation in 3 patients with dark complexion, whereas 12 out of 14 wheatish or brown complexioned had repigmentation after surgery and none of the 3 fair-complexioned patients had repigmentation.

Study Design and Patient Selection

The subjects for this study were selected from the outpatient Department of Periodontics, Sri Ramakrishna Dental College and Hospital, Coimbatore. 12 patients of age around 18 to 30 were selected based on inclusion and exclusion criteria mentioned below to undergo gingival depigmentation. All the patients were treated for gingival depigmentation using cryosurgery for upper arch and bur abrasion for lower arch.

Written consent was obtained from each patient prior to their inclusion in this study. Approval from Institutional Review Board (IRB) and Ethical Committee (EC) of Sri Ramakrishna Dental College and Hospital was obtained prior to implementation. Before undertaking any of the procedure, all the patients were reassured, supragingival ultrasonic scaling performed and oral hygiene instructions were advised.

Inclusion criteria

1. Diffuse continuous physiological pigmentation of the gingiva involving the facial aspect of anterior teeth
2. Patients with esthetic concern
3. Patients with good oral hygiene

Exclusion criteria

1. Smokers
2. Patients with compromised systemic health
3. Patients with cold intolerance
4. Patients under systemic medications

Armamentarium

Diagnostic Instruments

1. Mouth mirrors
2. Periodontal probe with William's markings
3. Tweezers

Surgical Instruments

1. Disposable syringes
2. 2% Lignocaine Hydrochloride with 1:80,000 adrenaline
3. Kidney Tray
4. High speed airotarhandpiece
5. Diamond abrasive points (Flame shaped FO-27, straight fissure TF-12, Tapered fissure TC-11. Dia Burs, Mani, Japan)
6. Sterile gauze pieces
7. Cheek retractor
8. Coe-Pak
9. K-Y Jelly
10. Minicryogun LNC 196 (Basco India)
11. Closed end cryoprobes 2 No's (3mm)
12. Liquid Nitrogen (-196°C)
13. Digital SLR camera (Nikon D90, 10.5 Megapixels)

Patients who were selected and gave their approval for gingival depigmentation were educated and motivated with more emphasis on proper oral hygiene maintenance. All selected patients were subjected to supragingival ultrasonic scaling.

All required clinical parameters were recorded at baseline for each patient and pre-operative photographs were also taken. The total surface area of pigmentation was measured with the help of 5 standardized photographs of each patient, preoperative, postoperative after 24 hours, 7 days, 30days, 90 days and 180 days, which was then calculated with help of a photo imaging software (Adobe CS 4) in which Grid lines were drawn for every 1sq cm and these were further divided by 10 thus providing with squares of 1 sq mm. By counting the number of squares which had pigmentation gave the total surface area of pigmentation.

Surgical Technique

Depigmentation using cryosurgery was performed in upper arch starting from canine to canine. Bur abrasion was done under local anaesthesia in relation to lower arch from canine to canine.

Cryosurgical procedure

The surgical site was cleaned by irrigating the area with saline. K-Y jelly was applied to improve thermal conductivity. The cryoprobe was placed on the gingiva, starting from the canine on the right side and the trigger was pressed. The probe tip began to freeze and adhere to the tissues as the probe cooled to - 196°C. The freezing was maintained for 20-30 seconds after which the trigger was released. The probe was allowed to thaw sufficiently before removing it from the applied site. Formation of an ice at the application site was observed.

This procedure was carried out until the entire desired area was covered with overlapping applications. A repeated cycle of application was done for 20 seconds in order to cover areas which would have missed during the first application.

Bur Abrasion

Local anesthesia was administered in relation to the surgical site, then gingival epithelium was abraded with flame shaped large bur involving the entire pigmented area extending from the free gingival margin to the mucogingival junction from the canine on right side extending up to the canine on the left side with the bur placed almost parallel to the long axis of the teeth with care taken not to expose the underlying bone. Bleeding was controlled by pressure pack and once homeostasis was achieved, the site was covered by periodontal dressing (Coe-Pak) for a week.

Estimation of pain intensity following surgery

Pain intensity was assessed using visual analogue scale (ANNEXURE I) where in the patients were asked to record and mark the severity based on four points "none, mild, moderate and severe" along with numbers assigned to each level as 0,1,2,3 up to 10. Where 0 indicates no pain and 10 indicates worst pain imaginable. Pain intensity of the patient was recorded 24 hours, 48 hours and 7 days post surgically.

Post Operative Protocol

Patients were instructed to continue with good oral hygiene and avoid trauma around the surgical site. Patients were prescribed with analgesics (Ibuprofen + Paracetamol) to be taken in case of unbearable pain and 0.2% chlorhexidine digluconate rinse twice daily for 2 weeks. Periodontal dressing was removed at the end of one week. Patients were recalled at the end of 24 hours, 7^{th}, 30^{th}, 90^{th} and 180^{th} day postoperatively for monitoring and reinforcement of oral hygiene.

Post Operative Instructions

1. Take rest on the day of surgery
2. After surgery if bleeding occurs from the treated area, place a clean wet gauze or cotton pack over the bleeding site and apply pressure for ten minutes
3. Should report to the Department of Periodontics immediately if bleeding persists
4. Avoid intake of any hot, hard food and do not disturb the operated area with the tongue
5. Should take the prescribed medicines as advised
6. Report in case dressing dislodges and care should be taken not to disturb the dressing
7. Avoid brushing the treated area for one week
8. To report for checkup as per schedule in the follow up program

ANNEXURE I

Sri Ramakrishna Dental College and Hospital

Department of Periodontics

PROFORMA

Case no : O.P. No:

Patients name :

Age :

Sex :

Address :

Phone No :

Chief Complaint :

Medical history :

	Upper Arch: Cryo Surgery	Lower Arch: Surgical Bur Abrasion
Pre operative: No. of squares with pigmentation		
7 days Post Operative No. of squares with Residual pigments		
No. of squares depigmented		

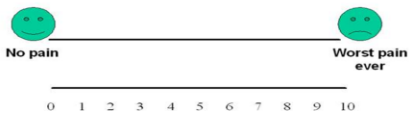
Post Operative Repigmentation.

	Upper Arch: Cryo Surgery	Lower Arch: Surgical Bur Abrasion
30 days		
90 days		
180 days		

Category and Numeric rating scale for measuring pain, Post operatively

(Visual Analogue Scale)

Upper Arch (Cryo surgery) 24 hours



Lower Arch (Bur abrasion) 24 hours

Upper Arch (Cryo surgery) 48 hours

Lower Arch (Bur abrasion) 48 hours

Upper Arch (Cryo surgery) 7 Days

Lower Arch (Bur abrasion) 7 Days

ANNEXURE II

Informed Patient Consent

Department of Periodontics

Sri Ramakrishna Dental College and Hospital

S.N.R Sons College Road, Coimbatore - 641006

I, the undersigned patient hereby consent to undergo treatment for depigmentation of gums using cryosurgery and surgical bur abrasion; I authorize the Department of Periodontics, Sri Ramakrishna Dental College and Hospital, Coimbatore to perform the above mentioned treatment.

I fully understand the procedure I will be undergoing and take sole responsibility in the event of any unforeseen complications.

Name: Place:

Signature: Date:

Statistical Analysis

The collected data was subjected to statistical analysis. Paired t test and one way variance analysis Anova was used to find the test of significance within the sample and between the samples. Study results were presented for the amount of depigmentation and repigmentation for a period of 180 days postoperative, and assessment of pain following surgery.

Armamentarium

Fig-1

Dewars Container with Liquid Nitrogen

Fig-2

Cryosurgery in relation to upper arch

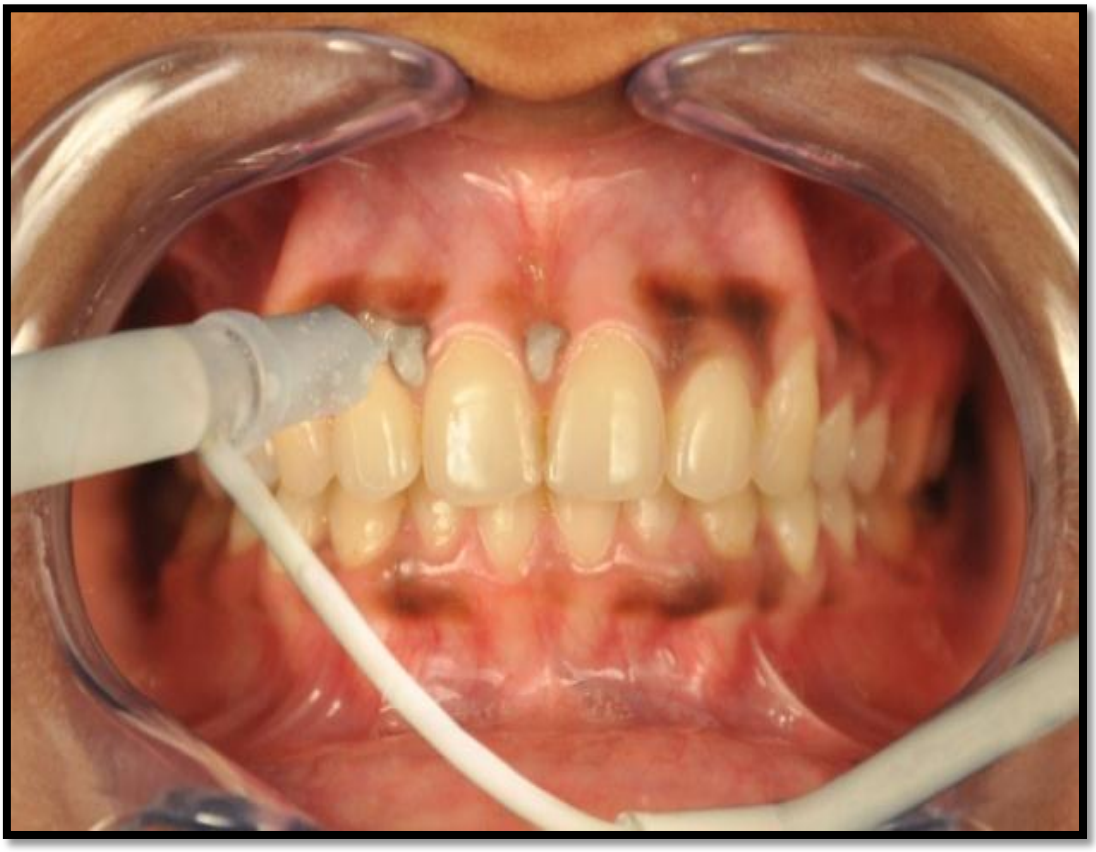


Fig-3

Bur abrasion in relation to lower arch

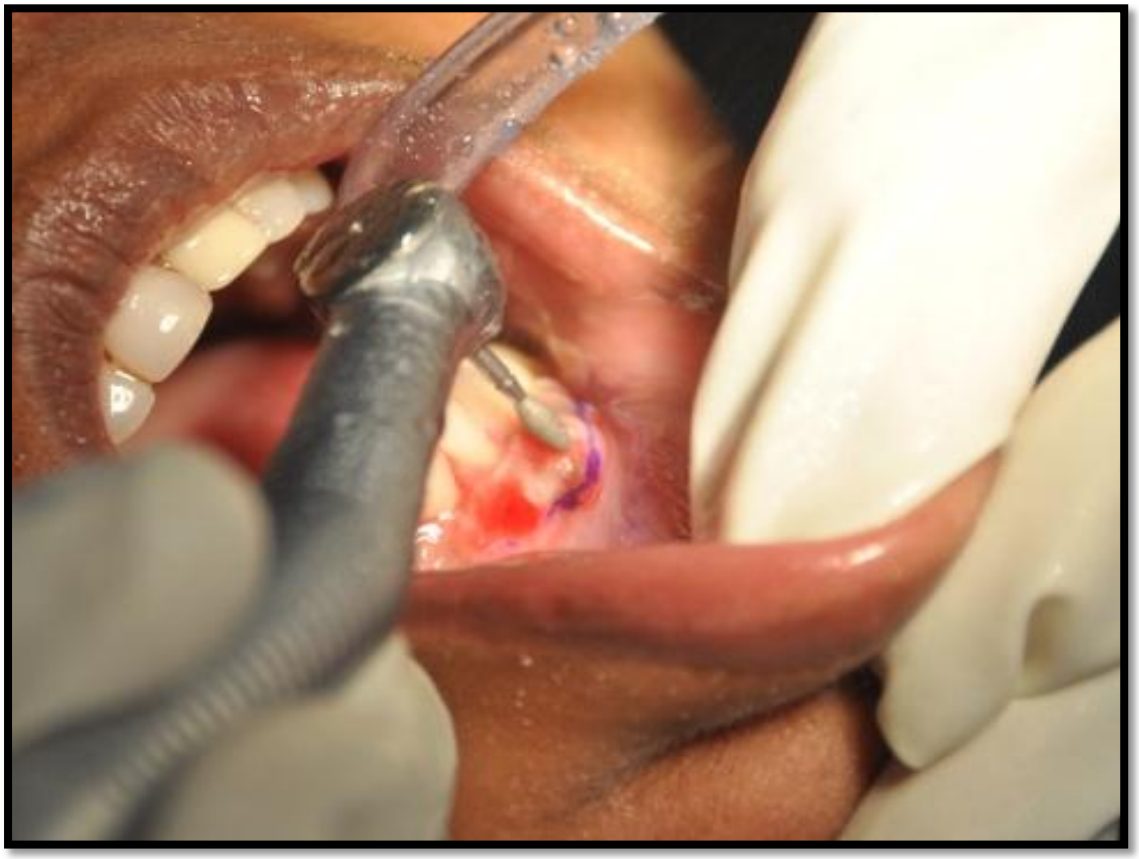


Fig-4

Grid lines drawn creating squares of 1 sq mm

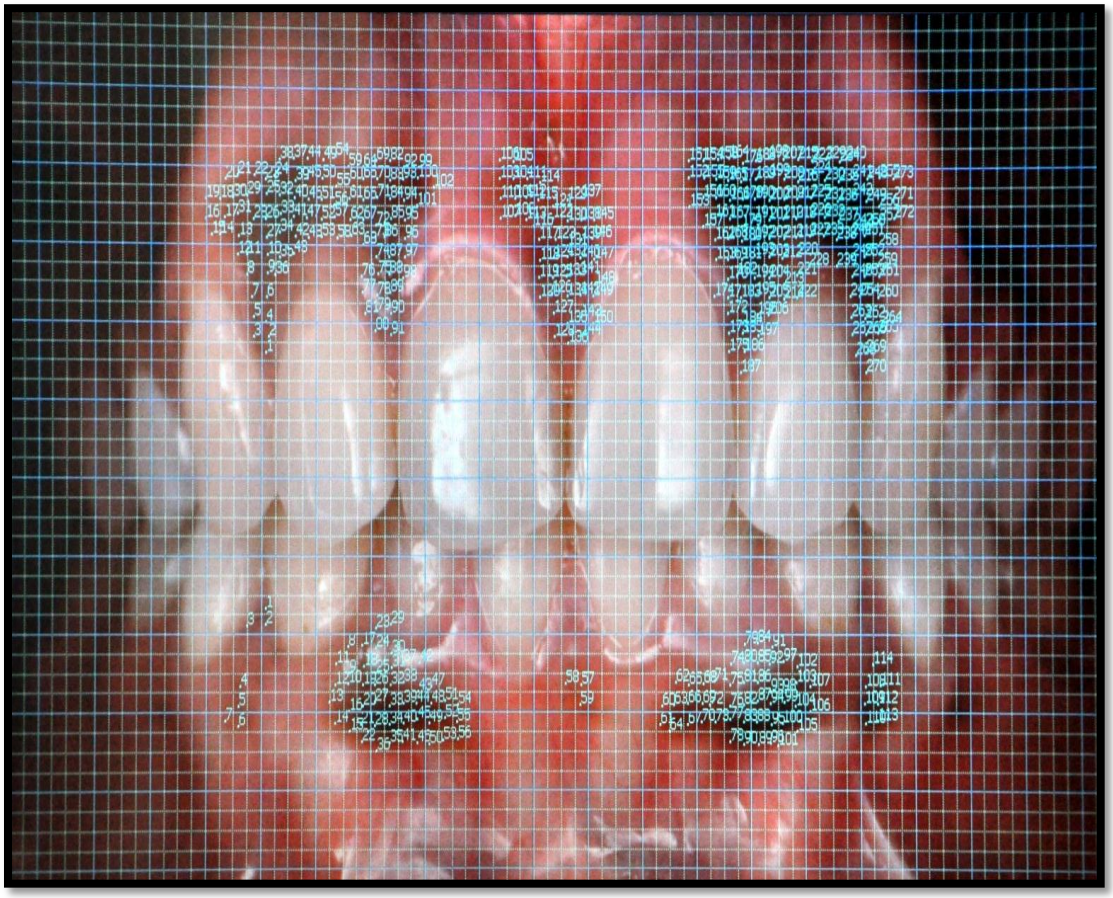


Fig-5

Baseline

Fig-6

After 24 Hours

Fig-7

After 7 Days

Fig-8

After One Month

Fig-9

After Three Months

Fig-10

After Six Months

Fig-11

12 patients between age 18 and 30 years from the outpatient Department of Periodontics, Sri Ramakrishna Dental College and Hospital, Coimbatore participated in the study. Patients were selected as per the inclusion and exclusion criteria described in the methodology.

A comparative study was designed in which upper arch was chosen for cryosurgery and lower arch was chosen for bur abrasion.

The collected data was subjected to statistical analysis. Paired t test and one way variance analysis Anova was used to find the test of significance within the sample and between the samples. Study results were presented for the amount of depigmentation and repigmentation for a period of 180 days postoperative.

General Findings

None of the patients complained of any discomfort during the cryosurgical procedure except for a slight tingling sensation as it was performed without local anesthesia. Patients did not develop any postoperative complications like bleeding, infection, swelling and sensitivity. On the other hand, pain experienced by patients with bur abrasion was more post operatively. It required administration of local anaesthesia and was associated with slight bleeding during the procedure.

Clinical Parameters

The total number of counted chambers at baseline in the maxilla were 384.83and for the mandible it was 202.92. The amount of pigmentation at baseline was more for the maxilla when compared to the mandible. This variance was due to difference in the surface area between the maxilla and the mandible. The comparison was significant as $p<0.001$. (Table-2) (Graph-1)

From Table-3, it can be inferred that the mean total of counted chambers in maxilla at baseline was 384.83. At the end of 7 days it was 0.00. At the end of one month it was 42.75, at the end of three months it was 121.58 and at the end of six months it was 220.08 (Graph-2). The mean total of counted chambers in maxilla at baseline was compared with 7days, one month, three months and six months. There was statistically significant difference between baseline to 7 days, baseline to one month, baseline to three months and baseline to six months $p<0.001$. (Table-4)

From Table-5, it can be inferred that the mean total of counted chambers in mandible at baseline was 202.92. At the end of 7 days it was 0.00. At the end of one month it was 16.92, at the end of three months it was 38.58 and at the end of six months it was 80.67 (Graph-3). The mean total of counted chambers in mandible at baseline was compared with 7 days, one month, three months and six months. There was statistically significant difference between baseline to 7 days, baseline to one month, baseline to three months and baseline to six months $p<0.001$. (Table-6)

The mean percentage of counted chambers (which represents repigmentation) in the maxilla at one month following depigmentation was 12.35%, similarly for the mandible it was 7.98%. There was no statistically significant difference between the maxilla and the mandible at 1 month. The comparison was not significant as $p=0.409$. (Table-7) (Graph-4)

The mean percentage of counted chambers in the maxilla at three months following depigmentation was 35.16%, similarly for mandible it was 19.36%. There was no statistically significant difference between the maxilla and the mandible at three months. The comparison was not significant as $p=0.068$. (Table-8) (Graph-5)

The mean percentage of counted chambers in the maxilla at six months following depigmentation was 59.58%, similarly for the mandible it was 42.13%. There was no statistically significant difference between the maxilla and the mandible at six months. The comparison was not significant as p=0.086. (Table-9) (Graph-6)

The mean pain experienced by patients at the end of 24 hours postoperatively in the maxilla was 1.17 and 2.25 for the mandible. There was no statistically significant difference in the pain experienced by patient between the maxilla and the mandible by the end of 24 hours. The comparison was not significant as p=0.012.(Table-10) (Graph-7)

The mean pain experienced by patients at the end of 48 hours postoperatively in the maxilla was 1.92 and 2.67 for the mandible. There was no statistically significant difference in the pain experienced by patient between the maxilla and the mandible by the end of 48 hours. The comparison was not significant as p=0.15. (Table -11) (Graph-8)

The mean pain experienced by patients at the end of 7 days postoperatively in the maxilla was 0.75 and 1.25 for the mandible. There was no statistically significant difference in the pain experienced by patient between the maxilla and the mandible by the end of 7 days. The comparison was not significant as p=0.30. (Table -12) (Graph-9)

Table-2

Mean total of counted chambers in the maxilla and the mandible at baseline

Group	N	Mean	Standard Deviation	't' Value	Level of significance
Maxilla	12	384.83	134	4.26	0.001
Mandible	12	202.92	62.95		

The comparison was significant as P<0.001

Table-3

Mean total of counted chambers in the maxilla at baseline, 7 days, one month, three months and six months

Group	N	Mean	Standard Deviation
Baseline	12	384.83	134
7 days	12	0.00	0.00
1 month	12	42.75	57.55
3 months	12	121.58	73.04
6 months	12	220.08	94.62

Table-4

Comparing the mean total of counted chambers in the maxilla at baseline with 7 days, one month, three months and six months

Group	N	Mean difference	Level of significance
Baseline – 7 days	12	384.83	0.001
Baseline – 1 month	12	342.08	0.001
Baseline - 3 months	12	263.25	0.001
Baseline - 6 months	12	164.75	0.001

The comparison was significant as P<0.001

Table-5

Mean total of counted chambers in the mandible at baseline, 7 days, one month, three months and six months

Group	N	Mean	Standard Deviation
Baseline	12	202.92	62.95
7 days	12	0.00	0.00
1 month	12	16.92	18.16
3 months	12	38.58	29.54
6 months	12	80.67	47.96

Table-6

Comparing the mean total of counted chambers in the mandible at baseline with 7 days, one month, three months and six months

Group	N	Mean difference	Level of significance
Baseline - 7 days	12	202.92	0.001
Baseline - 1 month	12	186.00	0.001
Baseline - 3 months	12	164.33	0.001
Baseline - 6 months	12	122.25	0.001

The comparison was significant as $P<0.001$

Table-7

Mean % of counted chambers in the maxilla and the mandible after one Month

Group	N	Mean	Standard Deviation	't' Value	Level of Significance
Maxilla	12	12.35	15.76	0.84	0.409
Mandible	12	7.98	8.68		

The comparison was not significant as $P=0.409$

Table -8

Mean % of counted chambers in the maxilla and the mandible after three months

Group	N	Mean	Standard Deviation	't' Value	Level of Significance
Maxilla	12	35.16	23.49	1.92	0.068
Mandible	12	19.36	16.23		

The comparison was not significant as P=0.068

Table -9

Mean % of counted chambers in the maxilla and the mandible after six months

Group	N	Mean	Standard Deviation	't' Value	Level of Significance
Maxilla	12	59.58	21.11	1.80	0.086
Mandible	12	42.13	26.11		

The comparison was not significant as P=0.086

Table-10

Pain assessment in the maxilla and the mandible after 24 hours

Group	N	Mean	Standard Deviation	't' Value	Level of Significance
Maxilla	12	1.17	2.25	1.60	0.12
Mandible	12	2.25	0.62		

The comparison was not significant as p=0.12

Table -11

Pain assessment in the maxilla and the mandible after 48 hours

Group	N	Mean	Standard Deviation	't' Value	Level of Significance
Maxilla	12	1.92	1	1.49	0.15
Mandible	12	2.67	1.44		

The comparison was not significant as p=0.15

Table -12

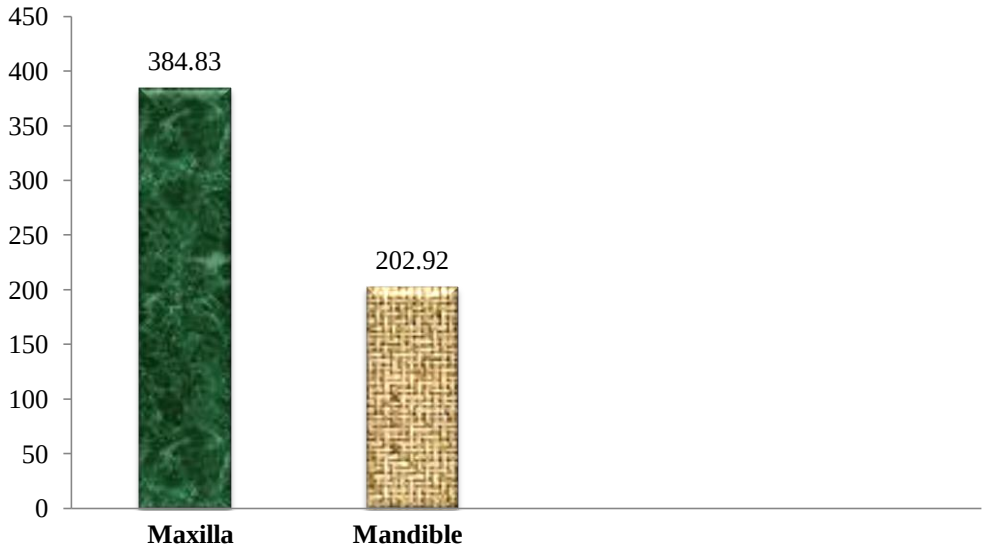
Pain assessment in the maxilla and the mandible after 7 days

Group	N	Mean	Standard Deviation	't' Value	Level of Significance
Maxilla	12	0.75	0.87	1.07	0.30
Mandible	12	1.25	1.36		

The comparison was not significant as p=0.30

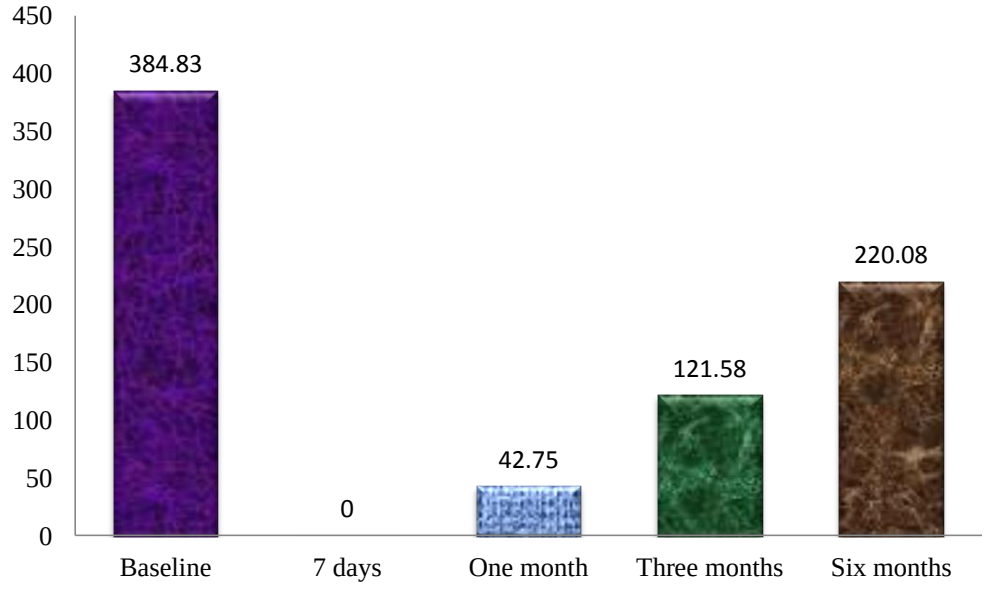
Graph-1

Mean total of counted chambers in the maxilla and the mandible at baseline



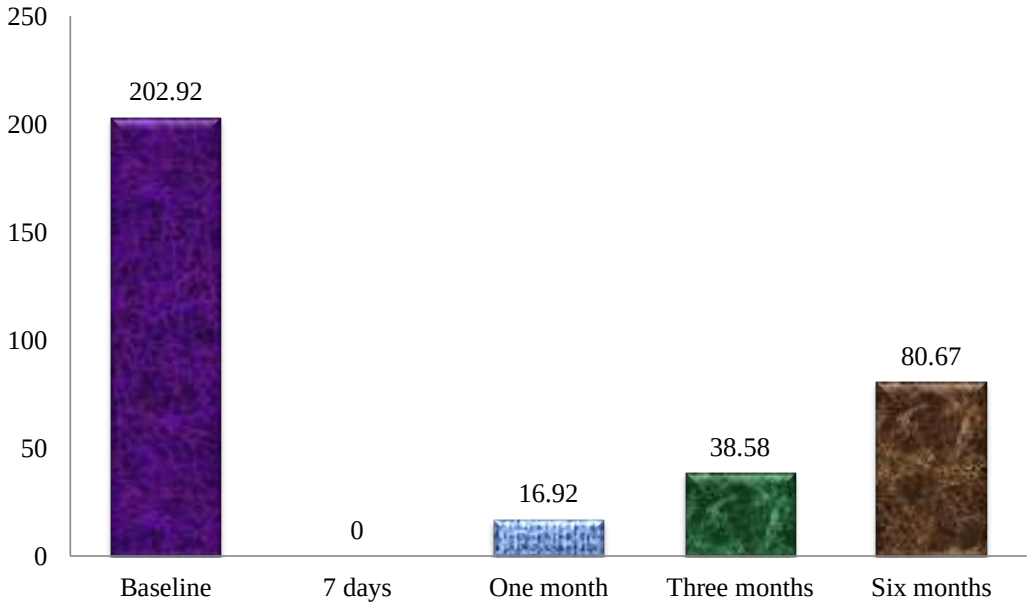
Graph-2

Mean total of counted chambers in the maxilla at baseline, 7 days, one month, three months and six months



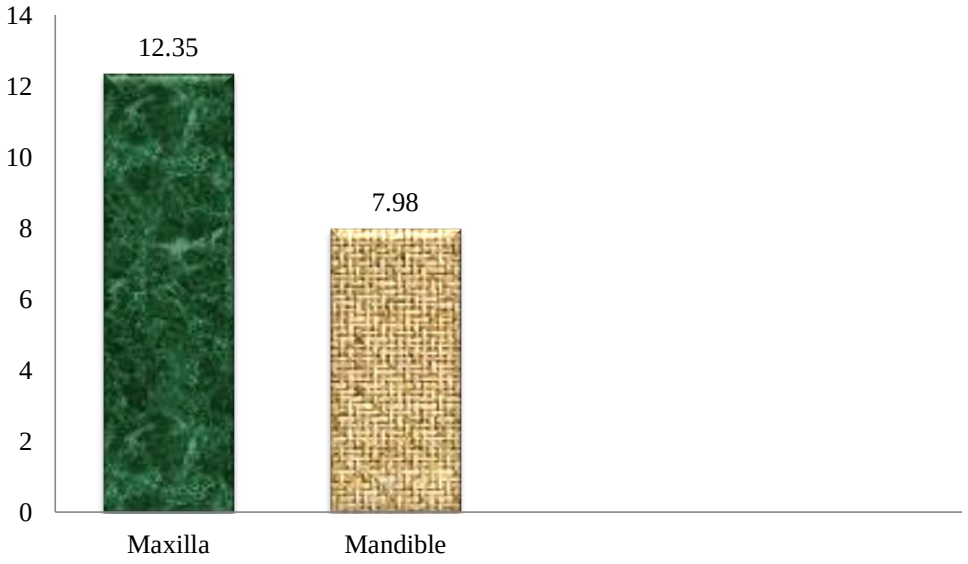
Graph-3

Mean total of counted chambers in the mandible at baseline, 7 days, one month, three months and six months



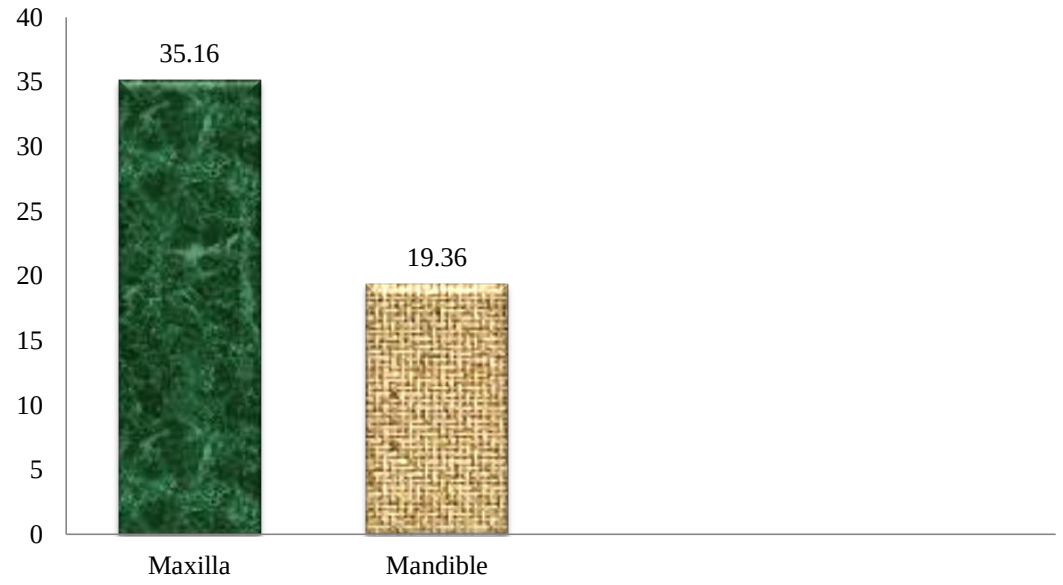
Graph-4

Mean % of counted chambers in the maxilla and the mandible after one month



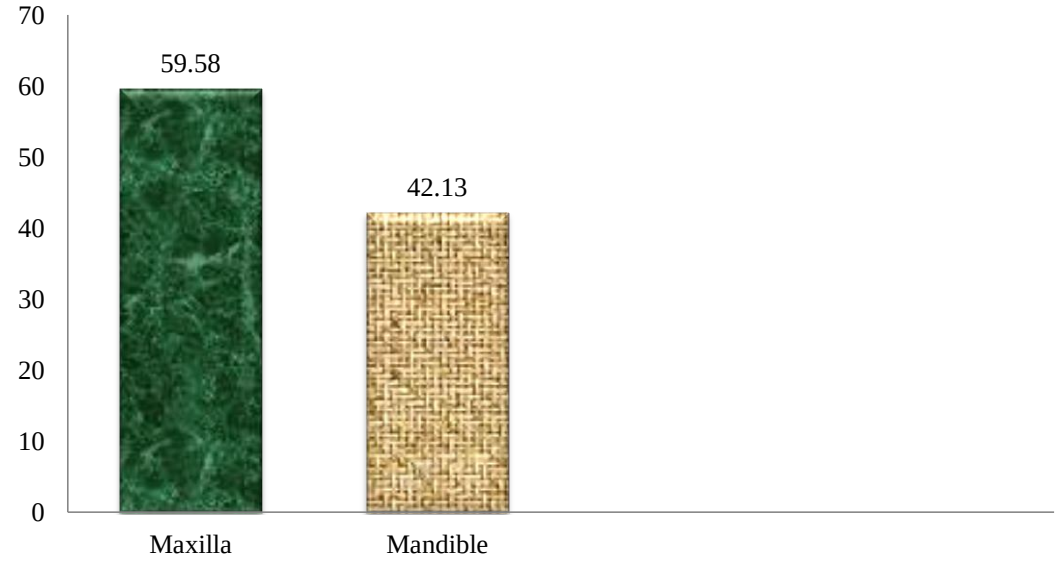
Graph-5

Mean % of counted chambers in the maxilla and the mandible after three months



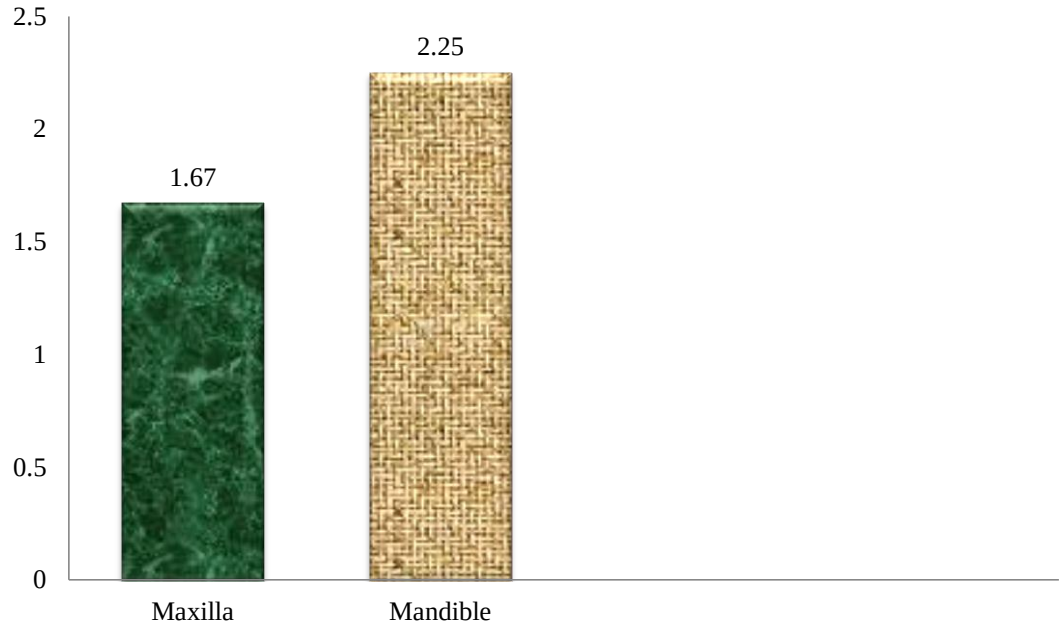
Graph-6

Mean % of counted chambers in the maxilla and the mandible after six months



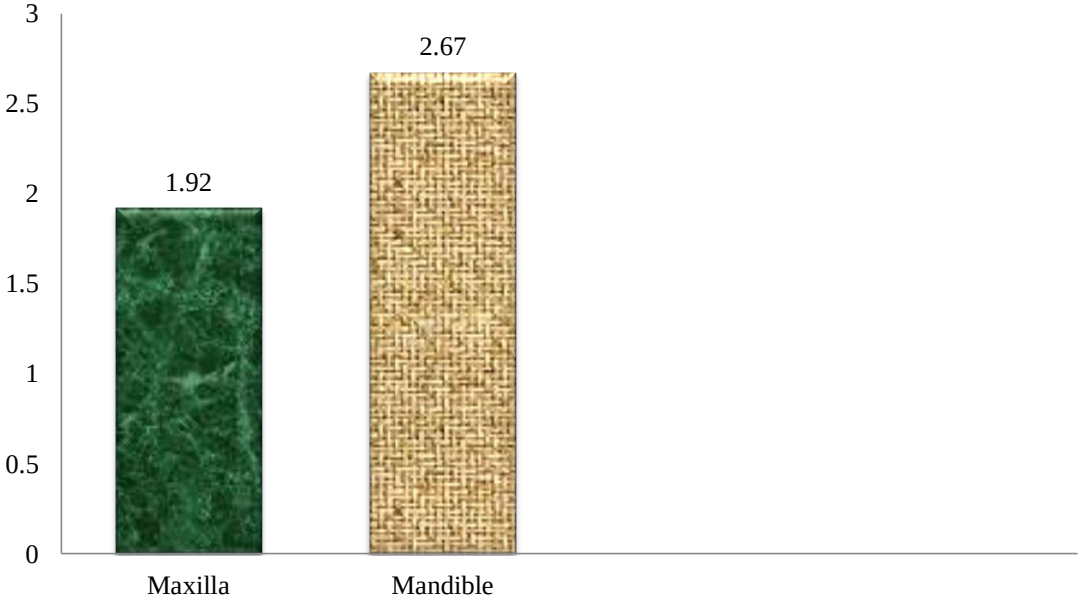
Graph-7

Pain assessment in the maxilla and the mandible after 24 hours



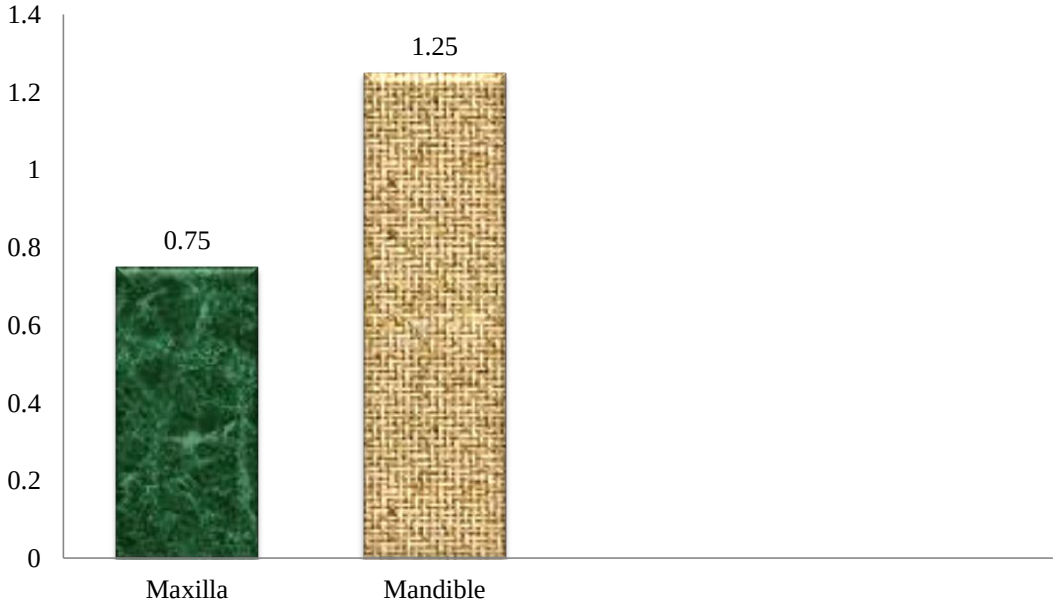
Graph-8

Pain assessment in the maxilla and the mandible after 48 hours



Graph-9

Pain assessment in the maxilla and the mandible after 7 days



This study was undertaken to determine the efficiency of cryosurgery and bur abrasion for the treatment of gingival pigmentation and to compare the efficiency of these two techniques and the rate of repigmentation.

Cryosurgery, an effective method of tissue destruction by freezing, is one of the established surgical techniques in medical and dental practice[79]. This technique has its own merits and demerits. Commenting on its merits, cryosurgery requires no anaesthesia, no hemorrhage occurs which reduces the chances of post operative infection and healing is also uneventful. Cryosurgical procedure was more acceptable to the patients as the procedure took less time.

On the other hand, cryosurgery has its own demerits too. It can leave behind residual pigments due to the inability to observe immediate changes after application of the cryoprobe. This could lead to areas unexposed to the cold temperature. This finding coincides with the study by **Yeh C-J**[82] (1998) who suggested a second course of cryosurgical treatment after 1 week to remove any residual pigments. It is also difficult to access the tip of the interdental papilla, as it is more narrow than the cryoprobe used. With cryotherapy, the depth and extent of destruction of the underlying tissue is difficult to assess, as reported by **Mayers PD et al**[51] (1971) in which he reported that cryogens can penetrate upto the superficial epithelial layers and rarely reaches basal and supra basal layers and does not reach the connective tissue. Cryosurgical procedure requires special container for storage of liquid nitrogen which is not easily available. Employing cryosurgical equipment also adds on to the expenses which is not routinely available in dental clinics and hospitals. The shelf life of liquid nitrogen is short and cannot be stored for long periods. Special care has to be exercised as accidental contact can cause injury to skin or other contacted areas.

Bur abrasion performed with a high speed handpiece and diamond bur was precise, definite and under control. With this technique, it was possible to appreciate the depigmented areas immediately and did not leave room for any residual pigments. This technique can be performed routinely in a regular dental clinic as it requires only a high speed handpiece and a diamond bur, unlike special equipments required for cryotherapy. This technique unlike cryotherapy is more painful, requires local anaesthesia and a periodontal pack. It leaves a raw wound, which is more painful as compared to cryotherapy. Great care should be taken while abrading epithelium which requires a feather touch movement. Excessive pressure can damage underlying connective tissue and /or periosteum which can cause bone exposure or can lead to gingival recession.

Assessment of gingival melanin pigmentation in the present study was done with the help of 6 standardized photographs and the number of chambers were counted with the help of grid tool using Adobe CS 4 software.

Postoperatively, repigmentation was seen in both maxillary and mandibular arch following depigmentation by the end of six months. The results obtained in the present study are in contrast with studies performed by **Tal et al**[76] (1987) who did not report repigmentation for 20 months following gingival depigmentation using cryosurgery. **Yeh C-J**[81] (1998) also showed no repigmentation after a follow up period of 2 years. In another study conducted by **Arikan F and Gurkhan A**[5] (2007) in which he used 1,1,1,2 tertrafluroethane and reported no repigmentation for a period of 30 months. Similarly another study conducted by **A. Darbandi** and **N. Amel Shahbaz**[1] (2004) performed gingival depigmentation using nitrogen oxide and reported no recurrence by the end of 6 months.

Another study conducted by **Shirazi AS et al**[73] (2010) in which depigmentation was performed with the help of liquid nitrogen cooled cotton swabs, reported no recurrence up to 12 months.

In our study postoperative repigmentation was reported following depigmentation using bur abrasion in all the 12 patients by the end of six months, but it was of less severity than cryosurgery.

The results of present study coincides with the findings reported by **Novaes AB Jr et al**[57] (2002) in which he performed gingival depigmentation using a Acellular Dermal Matrix Allograft on the right half of the maxilla and bur abrasion for the left half of the maxilla, reported repigmentation in left half of maxilla after 6 months. In another study, conducted by **Prasad D et al**[62] (2005) in which depigmentation was carried out using electrosurgery, bur abrasion and epithelial excision, repigmentation was reported in areas treated by bur abrasion with a follow up of 3 months.

Farnoosh AA[25] (1990) who performed gingival depigmentation with bur abrasion in 20 patients and reported slight repigmentation in two patients after 20 months of follow up. **Bishop K**[12] (1994) reported no repigmentation following depigmentation in one patient using bur abrasion over a period of one year. **Jayaprasad K and Chava V**[40] **(**1999) performed gingival depigmentation using scalpel and bur abrasion and reported repigmentation when followed up to 1 ½ year. **Mokeem SA**[53] (2006) performed gingival depigmentation in three patients using high speed handpiece and diamond bur and reported no repigmentation up to 18 months. Almost all the studies mentioned above were conducted in foreign countries.

The present study was conducted in South India which has predominantly more dark complexioned individuals. This may be one of the reasons for the reappearance of gingival melanin pigmentation while the incidence of repigmentation was minimal in the foreign studies. Since India is a tropical country, the exposure to actinic rays of the sun can also lead to pigmentation, as mentioned by **Dummett**[18] (1946). He claimed that since upper anteriors are more exposed to sunlight, the amount of pigmentation is also more. **Barrett AW and Scully C**[8] (1994) reported the differences in the melanin production and distribution in people with different race, Caucasians and African-Americans, wherein African-Americans demonstrated more melanin pigment when compared to Caucasians. **Holtzclaw D et al**[38] (2009) reported spontaneous repigmentation of non-pigmented palatal tissue, which according to him could be due to the release of endothelin-1 (ET-1) which is released upon blood vessel damage. It is hypothesized that use of bur abrasion can lead to damage of blood vessels and capillaries which in turn can lead to expression of endothelin-1 (ET-1). Another study conducted by **Kaur et al**[43] (2010) reported repigmentation in 15 out of 20 patients over an observation period of 9 months after de-epithelialization using Kirkland's gingivectomy knife. Seven patients showed no repigmentation at all. They reported repigmentation in 3 patients with dark complexion, whereas 12 out of 14 wheatish or brown complexioned had repigmentation after surgery and none of the 3 fair-complexioned patients had repigmentation.

A period of six months follow up is inadequate and studies with larger sample size and longer follow up period are required in Indian population.

This study was conducted in 12 subjects, with an esthetic complaint of hyperpigmented gingiva on the facial aspect of the anteriors. The patients were selected from outpatient Department of Periodontics, Sri Ramakrishna Dental College and Hospital, Coimbatore.

A comparative study was designed in relation to upper anteriors and lower anteriors. Upper anteriors was chosen for cryosurgical procedure and the lower anteriors for bur abrasion. The total surface area of pigmentation was measured with the help of 6 standardized photographs of each patient, preoperative, postoperative after 24 hrs, 7^{th}, 30^{th}, 90^{th} and 180 days which was then calculated with help of a photo imaging software (Adobe CS 4).

Patients with compromised systemic health, smokers and poor oral hygiene were excluded for the study. Oral prophylaxis and oral hygiene instructions were given to all the patients before performing the procedures. The final results were statistically analyzed and significance evaluated. From the results obtained, the following conclusions were arrived at:

1. Cryosurgical procedure and bur abrasion technique are highly efficient for the treatment of gingival pigmentation.

2. Statistical comparison of both the techniques in terms of efficiency was not significant.

3. The faster rate of repigmentation may be because the study was conducted in people of South India, who are more towards dark complexion.

4. The pain assessment, showed no statistically significant difference between cryosurgery and bur abrasion, but clinically patients preferred cryosurgery over bur abrasion, as it required no local anesthesia and post operative discomfort was also very minimal.

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